transporter inhibitors (such as probenecid) present in the assay system. Organic-anion transporter inhibitors are often toxic to cells and also interfere with the activities of bioactive compounds to be screened. In conclusion, Cal 520 AM is an improved fluorescent indicator for the measurement of intracellular calcium. The high signal-to-noise ratio and good intracellular retention properties make the Cal 520 AM a robust tool for evaluating GPCR and calcium channel targets as well as for screening their agonists and antagonists.

**1578-Pos Board B348**

**Studying Calcium Signal Reshaping by Buffers Observing the Competition of Two Dyes**

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Ca2+ signaling is ubiquitous across cell types. Ca2+ liberation through inositol 1,4,5-trisphosphate receptors (IP3Rs) is a key component of the Ca2+ signaling toolkit. The specificity and universality of intracellular calcium signals rely on the variety of spatio-temporal patterns that the concentration of this ion can display. It has been observed by I. Parker and collaborators that buffers can shape these patterns in different ways depending on their kinetics. In this work we study how these different effects may arise by observing IP3R-mediated Ca2+ signals using two dyes simultaneously. To this end we use a multi-spectral confocal microscope and two dyes (Fluo4 and Rhod2) that differ in their binding kinetics and emission spectra. In this way we are able to study the competition between the two dyes probing the effect of their different kinetics on the observed signals.

**1579-Pos Board B349**

**Intercellular Calcium Waves in Vascular Smooth Muscle Cells**

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Muscular arteries are able to actively modify their diameter by modulating the tone of the smooth muscle cells (SMCs) located within the arterial wall. The contractile state of the SMCs is regulated by cytosolic calcium transients and propagates as a wave over a significant distance along the vessel.
We studied the intercellular calcium wave propagation in primary cultured SMCs from rat mesenteric arteries. By using photolithography technique, two types of in vitro cells networks were developed: lines and loop patterns. We have recorded and analyzed calcium response and membrane potential variations for each cell, induced by single cell mechanical stimulus. The loop network was more efficient in transmitting the calcium signals from cell to cell. The calcium wave propagation through gap junctions was spatially limited in line patterns. Longer distance in calcium propagation was obtained in the presence of angiotensin II.
This study provides new experimental data supporting the idea that mechanical stimulation evokes a membrane potential depolarization which propagates to neighboring cells. The electrical depolarization is followed by a fast calcium entry that triggers calcium release from intracellular stores. In addition, these results suggest that increasing the expression of the gap junction protein connexin 43 by angiotensin II treatment, facilitates longer distance propagation of calcium waves in primary cultured SMCs.

**1580-Pos Board B350**

**Sarcoplasmic Reticulum Calcium Leak in Normal and Dystrophic Skeletal Muscle**

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Under resting conditions, external calcium is known to enter skeletal muscle cells while calcium stored in the sarcoplasmic reticulum (SR) leaks into the cytosol. The nature of the pathways involved in the resting sarcolemmal calcium entry and in the SR calcium leak are still debated but several lines of evidence suggest that an up-regulation of these calcium fluxes occurs in Duchenne Muscular Dystrophy (DMD). We investigated here SR calcium permeation at resting potential and in response to depolarization in voltage-controlled skeletal muscle fibers from control and *mdx* mice, the murine model of DMD. Using the cytosolic calcium dye Fura2, we first demonstrated that the rate of calcium increase induced by CPA (cyclopiazonic acid) inhibition of SR $Ca^{2+}$-ATPases at resting potential is significantly higher in *mdx* fibers suggesting an elevated SR passive calcium leak. However, in these experiments, sarcolemmal calcium influx may contribute to the CPA-induced calcium increase and another series of experiments indicated that CPA-induced SR calcium leak was deeply modified in the absence of external calcium. Fibers were then loaded with the low affinity calcium dye Fluo5N-AM and dialyzed with 50 mM EGTA to measure intraluminal SR calcium changes. Depolarization pulses evoked voltage-dependent Fluo5N fluorescence decreases followed by a recovery phase which was inhibited by CPA, demonstrating that Fluo5N actually reports intraluminal SR calcium changes. Voltage-dependence and magnitude of depolarization-induced SR calcium depletion were found to be unchanged in *mdx* fibers but the rate of the recovery phase that followed depletion was found to be faster, suggesting a higher SR calcium reuptake capacity in *mdx* fibers. Finally, CPA-induced SR calcium leak at −80 mV was found to be significantly higher in *mdx* fibers. The elevated SR passive calcium leak may participate to the muscle degenerative process in *mdx* muscle.

**1581-Pos Board B351**

**Altered Skeletal Muscle Excitation Contraction Coupling in Dysferlinopathy**

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Limb Girdle Muscular Dystrophy Type 2B (LGMD2B) and Miyoshi Myopathy (MM) are caused by mutations in the dysferlin gene, but the role of dysferlin in healthy muscle and the changes that occur when it is mutated or absent are poorly understood. Previous work supported a role for dysferlin in sarcolemmal repair following laser wounding or other in vitro injuries. We study the response of A/J mice, which lack dysferlin, to injury by large-strain lengthening contractions in vivo. We find that dysferlin promotes normal recovery from this physiological injury but is not necessary for sarcolemmal repair. Consistent with this, immunofluorescence microscopic studies of healthy muscle, that we fixed and treated in a hot, mildly acidic solution to expose dysferlin's epitopes, show that dysferlin is primarily in transverse tubules (TT), not the sarcolemma as previously reported. Furthermore, TT are disrupted when skeletal muscle is injured physiologically, and disruption is much more extensive in A/J muscles than in controls. Studies of FDB myofibers in tissue culture also demonstrate the presence of dysferlin in TT. Brief exposure of control myofibers to hypoosmotic solutions damages TT, in a process dependent upon extracellular Ca2+. As in vivo, A/J myofibers are more extensively damaged by osmotic shock than controls; they are indistinguishable from controls when shocked in Ca2+-free medium, however. Thus Ca2+ may promote damage, rather than participate in dysferlin-dependent membrane repair, as previously reported. Our results suggest that the changes in TT following injury in vivo and in vitro are similar, that they require extracellular Ca2+, and that they are much more pronounced when dysferlin is absent. We propose that dysferlin is essential for the integrity of the TT of skeletal muscle, in maintaining this integrity during contraction and relaxation, and in repairing damaged TT following injury.

**1582-Pos Board B352**

**Caffeine Treatment and Depolarization Alter the Spatial and Temporal Characteristics of Calcium Sparks on Intact Amphibian Skeletal Muscle**

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Calcium sparks were recorded on intact skeletal muscle fibers of the frog using high time resolution confocal microscopy (x-y scan: 30 Hz, line-scan: 60 kHz). Sparks were elicited by 1 mmol/l caffeine or subthreshold depolarization to different membrane potentials (data presented for −60 mV). Both treatments increased the frequency of sparks and altered their morphology. Images were analyzed by custom-made computer programs. Both the amplitude (in $\Delta F/F_0$; $0.49 \pm 0.025$ vs. $0.29 \pm 0.001$; n = 22426 vs. 23714; mean ± SEM, $p<0.05$) and the full width at half maximum (FWHM, in μm; parallel with fiber axis: $2.33 \pm 0.002$ vs. $2.21 \pm 0.005$; perpendicular to fiber axis: $2.07 \pm 0.003$ vs. $1.88 \pm 0.004$) of sparks was significantly greater after caffeine treatment than on depolarized cells. On x-y scans 25.8% (caffeine) and 16.4% (depolarization) of detected sparks overlapped with another one on the previous frame. Center of signal mass of overlapping sparks travelled shorter distances between consecutive frames after caffeine treatment then after depolarization (in μm; $1.80 \pm 0.017$ vs.